should be discontinued, and calculations individualized for each procedure.

## 25

### A NOVEL APPROACH FOR EX-VIVO EXPANSION (EVE) OF NK CD3$^-$/CD16$^+$/56$^+$BRIGHT/DIM SUBSETS EXPRESSING INCREASED INHIBITORY RECEPTORS FROM CRYOPRESERVED/THAWED/EXPANDED/RECRYOPRESERVED/RETHAWED (CTECT) AND CRYOPRESERVED/THAWED/RECRYOPRESERVED/RETHAWED/EXPANDED (CTCTE) UMBILICAL CORD BLOOD (UCB) USING ANTI-CD3, IL-2, IL-7 AND IL-12 (AB/CY)

*Ayello, J.[1], Satwani, P.[1], van de Ven, C.[1], Simpson, L.L.[1], Kurtzberg, J.[2], Cairo, M.S.[1] 1. Children's Hospital of New York-Presbyterian, Columbia University, New York, NY; 2. Duke University, Durham, NC*

CD56$^{bright+dim+}$NK express killer-Ig-like receptors (KIR) and C-lectin (NKG2), and natural cytoxicity receptors (NCR) involved with tumor target recognition may play a role in ACI of malignancies (Farag et al, Blood 100:1935, 2002). We compared expansion, maturation and cell survival of CD3$^-$/CD16$^+$/56$^+$, CD56$^{bright/dim}$, KIR inhibitory and C-lectin NK subsets in UCB aliquots. Non-adherent cells were cultured in SF AIM-V alone or with anti-CD3 (50 ng/ml), IL-2 (5 ng/ml), IL-7 (10 ng/ml) and IL-12 (10 ng/ml) (AB/CY). The expanded population was analyzed for expression of NK subsets (CD94$^+$, CD16$^+$, CD56$^{bright+/dim+}$) and NK receptors (KIR3DL1, KIR2DL1/S1, KIR2DL2 and NKG2A) by flow cytometry using CD16, CD56, NKBL1, CD158a, CD158b, CD94 and NKG2A mAbs. Apoptotic markers were determined by presence of Annexin V and PI. A significant increase in CD16$^+$/CD56$^{bright\ +/dim\ +}$ and CD94$^+$/NKG2A$^+$ expression was seen in CTECT and CTCTE in AB/CY compared to AIM-V alone (CD16$^+$/56$^{bright}$/CTECT: 11 ± 1 vs 2.5 ± .3%, $p < .05$; CD16$^+$/CD56$^{dim}$: 46 ± 6 vs 27 ± 2%, $p < .03$; CD16$^+$/56$^{bright}$/CTCTE: 9 ± 2 vs 2 ± .2%, $p < .05$; CD16$^+$/CD56$^{dim}$: 40 ± 2 vs 27 ± 3%, $p < .01$; CD94$^+$/NKG2A$^+$: CTECT: 17 ± 3 vs 2 ± .9%, $p < .001$; CTCTE: 25 ± 2 vs 8 ± 3%, $p < .001$). Significant increases were seen in NK KIR receptors expression (CD56$^{bright}$/KIR3DL1$^+$ and CD56$^{dim}$/KIR3DL1$^+$) when AB/CY was compared to AIM-V (CTECT: 11 ± 0.7 vs .4 ± .07%, $p < .0001$; 4 ± 0.1 vs 1.5 ± 0.4%, $p < .003$; CTCTE: 7 ± 2 vs 1 ± .3%, $p < .05$; 8 ± 1 vs 3 ± .8%, $p < .01$, respectively), CD56$^{bright}$/KIR2DL1/S1$^+$ and CD56$^{dim}$/KIR2DL1/S1$^+$ (CTECT: 12 ± 1 vs 3 ± .4%, $p < .004$; 41 ± 2 vs 9 ± 1%, $p < .0002$; CTCTE: 6 ± .5 vs 2 ± .1%, $p < .05$; 22 ± 8 vs 5 ± 1, $p < .01$) and CD56$^{bright}$/KIR2DL2$^+$ and CD56$^{dim}$/KIR2DL2$^+$ (CTECT: 14 ± .8 vs 2 ± .2%, $p < .0002$ and 29 ± .9 vs 12 ± .4%, $p < .0001$; CTCTE: 14 ± 2 vs 2 ± .1%, $p < .05$ and 25 ± 7 vs 6 ± 1%, $p < .001$, respectively). To determine if the increase was secondary to increased cell survival, CTECT and CTCTE AB/CY expanded cultures showed minimal apoptosis compared to SF AIM-V (1.1 ± 3 vs 6 ± .5%, $p < .001$; 5 ± 1 vs 11 ± .6%, $p < .05$). No significant difference between CTECT and CTCTE modalities was seen except in CD94$^+$/NKG2A ($p < .05$) and CD56$^{bright}$/KIR2DL2$^+$ ($p < .05$). These data suggest that CD16$^+$/CD56$^{+bright/dim}$ NK subsets expressing increased KIR and C-lectin receptors can be EvE with AB/CY from CTECT or CTCTE UCB for possible use in ACI for DLI after UCBT. In-vitro functional and in-vivo xenotransplant animal studies are underway to further examine the cytolytic activity of these UCB NK subsets.

## 26

### EX VIVO EXPANSION OF IMMATURE AND MATURE T CELLS DERIVED FROM UMBILICAL CORD BLOOD (UCB)

*Staba, S.L., Crapnell, K.B., Hall, J.G., Reese, M., Kurtzberg, J. Duke University Medical Center, Durham, NC*

Introduction: Unrelated umbilical cord blood (UCB) is an effective source of allogeneic hematopoietic stem cells for transplantation therapy in patients lacking matched bone marrow donors. Opportunistic infection is the major cause of mortality post transplant, due in part to the immunologic naiveté of the UCB T-cells. We hypothesized that UCB derived immature and mature T-cells could be expanded ex-vivo, and utilized a patient-derived skin stromal layer with a supplemental cytokine cocktail to support the growth of bulk UCB cells. The long term goal is to provide UCB-derived adoptive immunotherapy in UCBT recipients. **Methods:** Patient derived skin fibroblasts were cultured in a media of IMDM, 10% fetal calf serum and 10% horse serum for 14 days, then irradiated to 5,000 cGy. Cryopreserved UCB cells were thawed and cultured on this stromal layer in the same media supplemented with a cytokine cocktail of interleukin-7 (IL-7) (10 ng/ml), flt-3 ligand (10 ng/ml), and stem cell factor (50 ng/ml). UCB cells were cultured with cytokines alone and skin alone as controls. As a 2nd phase, interleukin-2 (IL-2) was added at day 14 for further expansion, with cells cultured for an additional 14 days thereafter. Cell count, viability, and FACs analysis were performed. **Results:** Gating on CD3+, in cells cultured with both skin stroma and cytokines mean fold increases of 6 in CD4+, 11 in CD8+ and 34 in CD4+/CD8+ cells were seen. With cytokines alone, mean fold increases were 3, 4 and 10 respectively. Minimal expansion occurred with skin alone. With cytokines and IL-2, mean fold increases were 4 in CD4+, 4 in CD8+ and 10 in CD4+/CD8+ cells. Maximal expansion occurred with skin stroma, the cytokine cocktail and IL-2, with mean fold increases of 9 in CD4+, 11 in CD8+ and 53 in CD4+/CD8+ cells. Results are summarized in the table below. **Conclusions:** The expansion of T-cells from unfractionated, red blood cell depleted, cryopreserved UCB can be accomplished using a cytokine cocktail of IL-7, Flt 3 ligand and SCF over a patient derived, irradiated skin stromal layer. Maximal expansion occurs with the addition of IL-2 and was greatest in the immature CD4+/CD8+ T-cell subset. We hypothesize that this subset of T cells could serve as a target population for adoptive immunotherapy, however further testing will determine the optimal cellular target and conditions for ex vivo expansion and immunization. These cells could then be used to augment immune reconstitution after UCBT.

**Table.**

| Conditions | Mean Fold Expansion | | |
|---|---|---|---|
| | CD4+ | CD8+ | CD4+/CD8+ |
| Skin Alone (n = 8) | 0 | 0 | 0 |
| Cytokines (n = 10) | 3 | 4 | 10 |
| Cytokines + IL-2 (n = 5) | 4 | 4 | 10 |
| Skin + Cytokines (n = 10) | 6 | 11 | 34 |
| Skin + Cytokines + IL-2 (n = 5) | 9 | 12 | 53 |

## 27

### ISOLATION AND FUNCTIONAL INHIBITORY AFFECTS OF SPECIFIC PEPTIDES BOUND TO TYPE 2 DENDRITIC CELLS (DC2)

*Rosenthal, H.S., Jaye, D.L., Cherry, E., Waller, E.K. Emory University, Atlanta, GA*

Introduction: The impact of immunoregulatory type 1 (DC1) and type 2 (DC2) dendritic cells in allogeneic hematopoetic progenitor cell (HPC) transplantation has increased in interest. We have shown that greater numbers of DC2s in allogeneic HPC transplanted grafts has resulted in an increase in post-transplant relapse and a reduction of graft vs host disease (GVHD). Isolation of dendritic cell specific peptides thru phage display technology provides a method of studying the immunoregulatory affects of these cells and perhaps engineer grafts with anti-tumor affects. **Method:** Random phage peptide libraries were incubated overnight at 4° C with column enriched peripheral blood DC2 cells. Unbound phage was removed by washing. FACS sorting of the Lin-, CD123+, HLA-Dr+ cells, further purified the DC2 population. DC2 bound phage were removed from the cell surface, eluted and purified. Three rounds of this phage selection process (panning) were performed. A portion of the round 2 phage purification was incubated with enriched monocytes prior to incubation with DC2 cells to assist in eliminating phage that recognized common epitopes on both cell types. Flow cytometry identified individual phage clones isolated from plaque assays that were

highly DC2 selective. Selective clones were DNA sequenced revealing the random peptide insert. A 1-way MLR was performed with varying dilution of each amplified DC2 specific phage to demonstrate the cellular affects on allogeneic T-cell proliferation. **Results:** Plaque assays from the monocyte adsorbed or non-adsorbed linear random peptide library after the three rounds of panning revealed two consensus sequences in 76 of the 78 (97%) isolated clones that were DC2 selective and one sequence found twice (3%) that was non-DC2 selective. The employed circular random peptide library revealed no DC2 selective sequences from 15 isolated in clones from the monocyte adsorbed and non-adsorbed plaque assays. Preliminary MLR data shows a 35 % reduction in allogeneic T-cell proliferation with the DC2 specific phage compared to control phage. **Conclusions:** Data shows that phage display technology can result in isolating highly specific DC2 peptides from a library of 10,000 different phage clones. Preliminary data suggests that binding DC2 specific peptides may inhibit the function of these immunoregulatory cells, leading to enhanced anti-tumor affect of the transplanted donor graft product by shifting it towards an activated Th1 immune response.

## GVH/GVL

### 28

### TREATMENT WITH GRANULOCYTE COLONY-STIMULATING FACTOR AFTER ALLOGENEIC BONE MARROW TRANSPLANTATION FOR ACUTE LEUKEMIA INCREASES THE RISK OF GRAFT-VERSUS-HOST DISEASE AND DEATH

*Ringdén, O.[1], Labopin, M.[2], Gorin, N.C.[2], Le Blanc, K.[1], Rocha, V.[3], Gluckman, E.[3], Reiffers, J.[4], Arcese, W.[5], Vossen, J.M.[6], Jouet, J.-P.[7], Cordonnier, C.[8], Frassoni, F.[9] 1. Centre for Allogeneic Stem Cell Transplantation and Division of Clinical Immunology, Huddinge Univ. Hospital, Stockholm, Sweden; 2. Hopital Saint-Antoine and EBMT Data Center, Institut des Cordeliers, Paris, France; 3. Hopital Saint-Louis, Paris, France; 4. GRP Hopital de Haut-Levèque, Pessac, France; 5. University La Sapienza, Rome, Italy; 6. BMT Centre, Leiden University Hospital, Leiden, Netherlands; 7. Service des Maladies du Sang, Hospital Claude Huriez, Lille, France; 8. Hospital Henri Mondor, Creteil, France; 9. Ospedale San Martino, Genova, Italy*

**Purpose:** Granulocyte colony-stimulating factor (G-CSF) is given after bone marrow transplantation (BMT) to shorten the neutropenic phase. Its effects have not been evaluated in a large patient population. **Patients and Methods:** We studied 1789 patients with acute leukaemia receiving BMT and 434 patients receiving peripheral blood stem cells (PBSC) from HLA-identical siblings from 1992 to 2002 and reported the findings to the European Group for Blood and Marrow Transplantation (EBMT). Among the BMT and PBSC patients, 501 (28%) and 175 (40%), respectively, were treated with G-CSF during the first 14 days after the transplant. The outcome variables were entered in a Cox proportional hazard model. **Results:** BMT and PBSC patients treated with G-CSF had a faster engraftment of absolute neutrophils $>0.5 \times 10^9$/l ($p < 0.01$), but platelet engraftment ($>50 \times 10^9$/l) was slower ($p < 0.001$). In the BMT patients, acute graft-versus-host disease (GVHD) grades II-IV was 50 ± 5% (±95% confidence interval) in the G-CSF group vs. 39 ± 3% in the controls (relative risk (RR) 1.33, p = 0.007, in the multivariate analysis). The incidence of chronic GVHD was also increased (RR 0.29, p = 0.03).

G-CSF was associated with an increase in transplant-related mortality (TRM) (RR 1.73, p = 0.00016), had no effect on relapse, but reduced the survival (RR 1.7, $p < 0.0001$) and leukaemia-free survival rates (LFS) (RR 1.55, p = 0.0003). No such effects of G-CSF were seen in patients receiving PBSC. **Conclusion:** After BMT, platelet engraftment was delayed, and GVHD and TRM were increased. Survival and LFS were reduced. This suggests that G-CSF should not be given shortly after BMT.

### 29

### SUBEROYLANILIDE HYDROXAMIC ACID REDUCES ACUTE GRAFT-VERSUS-HOST DISEASE AND PRESERVES GRAFT-VERSUS-LEUKEMIA EFFECT BY INHIBITING HISTONE DEACETYLATION

*Reddy, P.[1], Maeda, Y.[1], Hotary, K.[1], Dinarello, C.A.[2], Ferrara, J.L.[1] 1. University of Michigan Comprehensive Cancer Center, Ann Arbor, MI; 2. University of Colorado Medical Center, Denver, CO*

Pro-inflammatory cytokines and the loss of gastrointestinal tract integrity contribute to acute graft-versus-host disease (GVHD) whereas the donor cytotoxic responses are critical for graft-versus-leukemia (GVL) preservation. Suberoylanilide hydroxamic acid (SAHA) is an anti-tumor agent that inhibits the activity of histone deacetylases (HDAC) and reduces the production of proinflammatory cytokines. Using a well characterized allogeneic murine BMT model B6 (H-$2^b$) → B6D2F1 (H-$2^{b/d}$) we studied the effects of HDAC inhibition by SAHA on acute GVHD. Recipients were transplanted with $2x10^6$ donor T and $5x10^6$ BM cells after13 GY TBI. Intra-peritoneal injections of 35 mg/kg/day of SAHA from days +3 to day +7 increased histone H3 acetylation in splenocytes harvested 7 days after BMT, confirming the inhibition of HDAC enzymes. SAHA treatment significantly reduced the serum levels of pro-inflammatory cytokines such as TNF-a, IL-1β and IFN-γ ($P < 0.04$) in the allogeneic recipients on day +7 after BMT. Intracytoplasmic staining by flow cytometry and RPA analysis of the host splenocytes on day +7 confirmed the decrease in the cytokine protein and mRNA. SAHA significantly improved the survival ($P < 0.002$) and reduced intestinal damage from GVHD of the allogeneic recipients . However SAHA did not suppress the donor T cell expansion in vivo and the proliferative and cytotoxic responses to host antigens in vitro measured 7 and 14 days after BMT. To test the effect of SAHA on GVL effects, recipients were injected with lethal doses of P815 (H-$2^d$) tumor cells at the time of BMT. SAHA treatment resulted in significantly improved leukemia-free survival after allogeneic BMT ($P < 0.05$) whereas all the syngeneic BMT recipients of SAHA died of tumor ruling out direct anti-tumor effects of SAHA. Furthermore SAHA increased H3 acetylation in the splenocytes from both the syngeneic and allogeneic leukemic recipients on day +7, confirming that inhibition of HDAC enzymes alone is not sufficient for leukemia free survival in this system. We also tested the effect of SAHA in a second allogeneic BMT model (BALB/c → B6), where it also significantly improved survival ($P < 0.001$) and preserved GVL effects when recipient mice were injected with lethal doses of EL-4 (H-$2^b$) tumor ($P < 0.04$). We conclude that HDAC inhibition regulates acute GVHD in these models and suggest that this class of pharmacologic agents may provide a novel strategy to reduce GVHD while maintaining the beneficial GVL effects.

### 30

### ABROGATION OF THE INTERACTIONS BETWEEN CXCR3 AND ITS LIGANDS MIG AND IP-10 REDUCES THE SEVERITY OF IDIOPATHIC PNEUMONIA SYNDROME AFTER ALLOGENEIC STEM CELL TRANSPLANTATION

*Hildebrandt, G.C.[1], Corrion, L.C.[1], Olkiewicz, K.M.[1], Liu, C.[2], Cooke, K.R.[1] 1. Dept. of Pediatrics; BMT Program, University of Michigan, Ann Arbor, MI; 2. Dept. of Pathology; University of Florida School of Medicine, Gainesville, FL*

Chemokines are important mediators in the development of Idiopathic Pneumonia Syndrome (IPS), a major cause of mortality after allogeneic (allo) stem cell transplantation (SCT). We hypothesized, that recruitment of donor T cells to the lung is dependent, at least in part, upon interactions between the chemokines MIG and IP-10 and their receptor CXCR3. CXCR3 is expressed on activated T cells; MIG and IP-10 can be induced in various cell types by IFNγ alone or in combination with TNFa or IL-1ß. We tested this hypothesis using an established murine SCT model wherein lethally irradiated bm1 mice receive SCT from either syngeneic (bm1) or allogeneic (B6Ly5.2) donors. MIG and IP-10 BAL levels were significantly elevated in allo recipients compared to syn controls at weeks 1 (MIG: 162.8 ± 37.6 vs 0; IP-10: 41.1 ± 4.2 vs 0 pg/ml) and 4 (MIG: 153.5 ± 41.7 vs 21.6 ± 9.0; IP-10: 202.0 ± 61.1 vs 3.8 ± 0.9 pg/ml) and correlated with the infiltra-